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14. ABSTRACT
By facilitating the structural maturation and thereby the stability and activity oncogenic proteins, such as ErbB2, the molecular chaperone Hsp90 has emerged as a promising cancer therapeutic target. Toward understanding the regulation of Hsp90 and identifying new therapeutic approach targeting Hsp90 activity, we have characterized reversible acetylation as a critical mechanism that regulates Hsp90 function. Here we present evidence that Hsp90 chaperone activity is regulated by reversible acetylation and controlled by the deacetylase HDAC6. Inactivation of HDAC6 leads to Hsp90 hyperacetylation, its dissociation from an essential co-chaperone, p23, and a loss of chaperone activity. Using glucocorticoid receptor (GR) as a model client protein, we showed that in HDAC6 deficient cells, Hsp90-dependent maturation of the glucocorticoid receptor (GR) is compromised, providing evidence that HDAC6-catalyzed deacetylation is critical for Hsp90 activity. Our study identifies reversible acetylation as a unique mechanism that regulates Hsp90 chaperone complex activity. Based on this observation, we are now investigating whether HDAC6-regulated Hsp90 acetylation is also critical for ErbB2-induced tumor transformation.

15. SUBJECT TERMS
p53, acetylation, ubiquitination,MDMD2, HDAC

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Title: A novel deacetylase (HDAC6)-Hsp90 complex in the regulation of EGFR and ErbB-2 signaling in breast cancer

Introduction:

The long-term goal of this research proposal is to test the hypothesis that HDAC6-regulated acetylation plays a critical role in Hsp90-dependent ErbB2 oncogenic kinase-induced tumor transformation and thereby establish HDAC6 as a therapeutic target for treating breast tumor caused by ErbB2.

While gene amplification is the causative event underlying over-expression of ErbB2, the stability and activity of this oncoprotein critically depends on the molecular chaperone heat shock protein 90 (Hsp90). In conjunction with specific cofactors, termed co-chaperones, Hsp90 is believed to facilitate the proper folding of mutated, chimeric or over-expressed oncogenic proteins such as ErbB2, thereby promoting malignant transformation. Supporting this view, Hsp90 is abnormally up-regulated in many human tumors including breast cancers, and specific Hsp90 inhibitors, such as 17-allylamino-17-demethoxygeldanamycin (17-AGG), induce destabilization of ErbB2 and show anti-tumor activity against human breast cancer in murine xenograft models {Solit, 2003 #35}. While Hsp90 has emerged as a promising target in cancer treatment, little is known about how its activity is regulated to facilitate ErbB2-mediated oncogenesis. Understanding this process could provide valuable information for developing a mean to disrupt such regulation and thereby impair ErbB2-dependent tumorigenesis. Here we have identified HDAC6-regualted reversible acetylation as a critical mechanism that regulates Hsp90 chaperone activity. We demonstrated that HDAC6 is a Hsp90 deacetylase. Furthermore, using a model Hsp90 client protein glucocorticoid receptor, we demonstrated that HDAC6 is required for full Hsp90 chaperone activity. This observation provides strong rationale to study the potential role for HDAC6 in ErbB2-induced tumor transformation. Indeed, our preliminary study supports the idea that HDAC6 is required for ErbB2-tumor transforming activity.

Body:

1. **Hsp90 chaperone activity toward glucocorticoid receptor is defective in cells deficient in HDAC6 (see attached manuscript)**
2. **HDAC6 is required for ErbB2-dependent signaling and efficient proliferation in a xenograft model.**

1. In the previous report, we have showed that Hsp90 becomes hyperacetylated in HDAC6 deficient cells. The hyperacetylation of Hsp90 is correlated with a defect in the maturation of glucocorticoid receptor (GR). In the current study, in collaboration with Dr. William Pratt's group, we now provided the critical evidence that Hsp90 produced in HDAC6 deficient cells is in fact defective in supporting in vitro GR maturation, and most importantly, this deficiency can be reversed by wild type Hsp90 purified from wild type cells.

2. As ErbB2-maturation also depends on Hsp90, we test the hypothesis that HDAC6-mediated deacetylation is important for Hsp90 to efficiently facilitate ErbB2-depenent oncogenesis. In the last report, we shows that inactivation of HDAC6 by small interference RNA markedly reverses the transformed phenotype of SKBR3, an ErbB2-overexpressing human breast cancer cell line (Figure 1). Now we have obtained evidence biochemical evidence that erbB2-dependent erk Kinase phosphorylation is defective in HDAC6 deficient cells. Although our study on ErbB2 activity remains inconclusive at the present, the Erk phosphorylation defect provides one potential molecular basis for the observed defects in transformation (Figure 2). Furthermore, in the process of analyzing receptor kinase-ErkK pathway, we found that the levels of Raf-1 and B-raf kinases, both are Hsp90 client proteins critical for transformation, are reduced in HDAC6 deficient cells. Together, these observations suggest that HDAC6 is important for Hsp90 dependent client kinase signaling and transformation (Figure 3). We are in the process of assessing whether loss of HDAC6 affecting ErbB2-dependent tumor formation in a xenograft model.

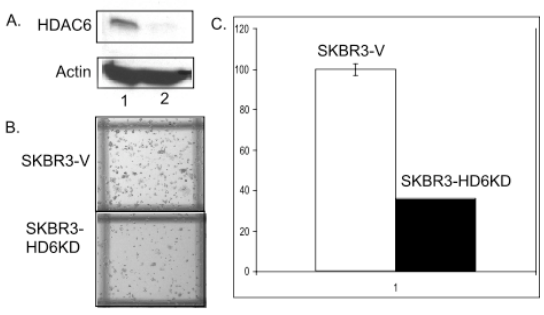

Figure 1. Anchorage independent growth is impaired with loss of HDAC6 in SKBR3 cells. (A) HDAC6 knockdown SKBR3 cells show a significantly lower level of HDAC6. Whole cell lysate from vector control (lane1), and HDAC6 knockdown SKBR3 cells (lane2) were immunoblotted for HDAC6 and total actin. (B) Representative images of soft agar plate. Fifty thousand SKBR3 cells stably expressing a siRNA for HDAC6 (SKBR3-HD6KD) or vector (SKBR3-V) were plated in 0.3% soft agar with regular media for three weeks. (C) Quantification of colony formation in soft agar

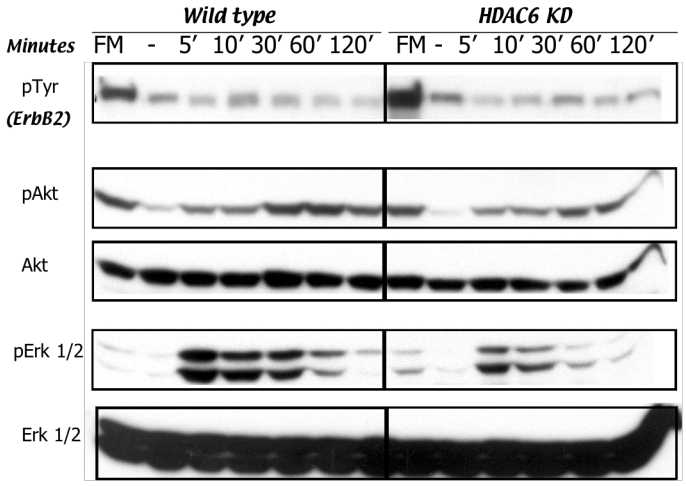

Figure 2. Wild type or HDAC6 knockdown skbr3 cells are cultured in full medium (FM), of serum starved followed by EGF stimulation. Note that phospho-ErbB2 (pTyr), phospo-AKT (pAKT) appear to be comparable in wild type and HDAC6 KD cells. However, phospho-Erk1/2 (p-Erk1/2) is dramatically reduced in HDAC KD cells.

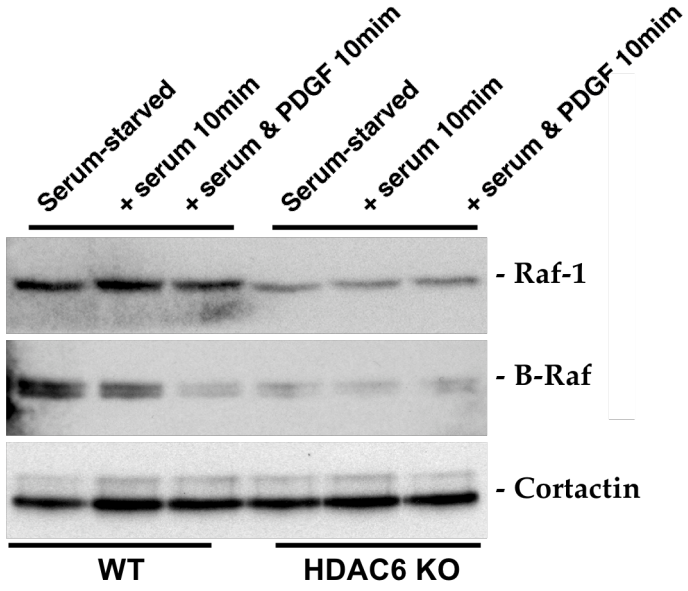

Figure 3. Wild type or HDAC6 deficient cells are serum starved or treated with serum or PDGF as indicated. Note that the protein level of raf-1 and B-Raf are significantly reduced in HDAC6 deficient cells.

Key Research Accomplishment

1. We have identified HDAC6 as a Hsp90 deacetylase and show that Hsp90 acetylation negatively regulates its chaperone activity by dissociating its essential co-factor.
2. We have established the preliminary result that HDAC6 is required for Hsp90-dependent ErbB2 oncogenic activity and identified another potential defect in raf-1 and B-Raf kinase stability.

Reportable Outcome:

Murphy P.J, Morishima Y, Kovacs J.J, **Yao T.-P**, and Pratt WB. Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. **J. Biol. Chem.** 280(40):33792-33799 (2005).

Kovacs, J.J., Cohen T and **Yao, T.-P.** Chaperoning steroid hormone signaling via reversible acetylation. Nucl Recept Signal. 3:e004 (2005) (review article)

Conclusion:

Our study has provided strong evidence that Hsp90 chaperone activity is regulated by reversible acetylation controlled by HDAC6. This result provides strong rationale to investigate the role for HDAC6 in ErbB2-dependent oncogenic transformation.